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Biomarkers of Fatigue: Ranking Mental Fatigue Susceptibility

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Biosciences and Performance Division

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This is the mental fatigue susceptibility ranking portion of a study seeking to determine metabolic biomarkers for resistance to fatigue. A total of 23 paid subjects completed a fatigue protocol involving informed consent, medical screening, Tuesday and Wednesday evening training on cognitive tasks, providing five urine samples (beginning with a Friday morning baseline), refresher training, and an experimental session (with six, four hour long blocks) starting Friday evening. Subjects showed the expected declines in performance during the 36-hour, 15-minute period of sleep deprivation without caffeine. The simple change from baseline results on the Psychomotor Vigilance Task (PVT) is recommended as the primary classifier for mental fatigue susceptibility. The next part of the study will use nuclear magnetic resonance (NMR) spectroscopy, liquid chromatography (LC), and mass spectrometry (MS) of the urine samples to determine if biomarkers are indicators of mental fatigue susceptibility.

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TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

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1.0 INTRODUCTION

This report describes part of a research agenda investigating the relationship between urine metabolomics and fatigue caused by sleep deprivation. It is hoped that urine metabolomics can either index or predict such fatigue impact. The research agenda consists of two independently executed parts. The first part, conducted at Brooks City-Base TX, is a fatigue study in which study participants are ranked on their fatigue susceptibility, based on their behavior observed during sleep deprivation. Concurrent to collecting this behavior data, urine samples and food intake logs from the same participants were collected. The second part of the agenda, to be conducted by personnel in the Applied Biotechnology Branch (711 HPW/RHPB), Wright Patterson AFB, OH, is an analysis that will correlate the urine samples to the rankings of participant fatigue susceptibility. Nuclear magnetic resonance (NMR) spectroscopy, liquid chromatography (LC), and mass spectrometry (MS) will be performed on the samples. Principal component analysis (PCA) and linear discriminant analysis (LDA) of the resulting spectra will be used to determine if there are biomarkers of the process. As the first part (ranking and urine collection) has been concluded, the current report provides the behavioral results enabling execution of the second part.

A heavily researched area in human performance is sleep deprivation (Harrison & Horne, 2000; Pilcher & Huffcutt, 1996), which is a common cause of mental fatigue. Mental fatigue is a highly undesirable and ever present consequence of long-duration and counter-circadian military operations; therefore, detecting and ameliorating mental fatigue is a high-priority issue in organizations that invest in human performance optimization (such as the U.S. Air Force). Commanders currently have no real-time biomarkers of mental fatigue and cannot identify in advance individuals who are particularly susceptible or resistant to the mental fatigue caused by sleep deprivation. The requirements for fatigue-robust military operations provide the justification for this research.

Fatigue susceptibility is not a random variable. Van Dongen, Baynard, Maislin, and Dinges (2004) discussed the stability of inter-individual differences in fatigue susceptibility seen after occasions observing human performance under two periods sleep deprivation. For the Psychomotor Vigilance Task (PVT, Dinges et al., 1997), a task measuring attentional lapses, Van Dongen et al. (2004) reported two studies in which the Intraclass Correlation Coefficient (ICC) for performance was highly significant (i.e., ICCs between 0.58 and 0.68). They also reported large ICCs for tasks requiring cognitive work, such as digit-symbol substitution (i.e., ICC of .82). The current study follows prior demonstrations that identified naïve participants (i.e., inexperienced with sleep deprivation studies) that differ in fatigue susceptibility. Because of budgetary and time constraints, we relied on only one sleep-deprivation observation. After two evenings of training, study participants were exposed to 36 hours-15 minutes of sustained wakefulness. During the last 24 hours-15 minutes, operational work was simulated using repeated cognitive tests. Once all the data was collected (n=23 completed the study), participants were ranked on their fatigue susceptibility based on how much their performance declined with sleep deprivation.

Urine samples were collected the morning the protocol started and during the sleep deprivation period. The urine samples were sent to Wright Patterson AFB for later analysis following every protocol run. Urine was targeted as a source of biomarkers with the promise for tracking mental fatigue because urine is a noninvasively-collected, information-rich biofluid that can be analyzed in powerful ways (e.g., nuclear magnetic resonance and liquid chromatography/mass spectrometry) to understand the underlying physiological state of the performer. Using these methodologies Wright Patterson AFB personnel will determine if there are metabolomic differences in the urinary profiles of fatigue resistant and fatigue susceptible participants.

2.0 PROCEDURE

2.1 Subject Inclusion Criteria

The protocol (F-WR-2010-0029-H) was approved by the Wright Patterson Institutional Review Board (IRB). Military and civilians from the local Brooks community were recruited by flyers posted at the fitness center and outside bathrooms in buildings at Brooks. Personnel who had participated in a previous sleep deprivation study were excluded from participation in this study.

The following is a list of Informed Consent Document (ICD) stipulated criteria for participation in the study. Our ability to verify some of these criteria were limited. For many criteria, we could only stipulate that the subject should comply. Age limits were 19-years old to 39-years old. Participants were required to be un-medicated throughout the protocol, including over-the-counter medications, herbal supplements, and vitamins. Participants were restricted to moderate habitual caffeine use (no more than the equivalent of three cups of coffee a day), with no tobacco use during the past 60 days. Neither caffeine nor nicotine in any form was allowed during the protocol. Each subject received up to $435.63 compensation for the study ($10.00/hour for 8-hours, 15-minutes training and refresher training, $12.50/hour for 24-hours, 15-minutes of the experimental session, and a $50 bonus for completing the study). Subjects who did not complete the study were compensated on a *pro rata* basis using the payment schedule.

Up to 30 days prior to the study, all subjects were told to maintain work hours within the time period from 0700 to 1800. They were also required to have a sleep/wake history (three days prior to the study, as verified by wrist-worn actigraphs signed out the Tuesday before the study) of seven hours per night minimum within the time window of 2300 to 0700 (+/- 1 hour on each end of the window). A similar sleep/wake history was required for the 30 days prior to the week of the study.

The week of the study some dietary, exercise, and caffeine restrictions went into effect. The full participant instructions can be found in Appendix A: Study Restrictions.

2.2 Experimental Design

A 12-level within-subjects factor (trial/fatigue) was crossed with a two-level between-subjects "group" factor. The group factor is determined by which particular tasks are trained or untrained with respect to particular subjects once sleep deprivation was initiated. Note that tests considered in the ranking of participants were always trained the same amount for all participants. These tests were the ANAM (Automated Neuropsychological Assessment Metrics, Reeves, Winter, Kane, Elsmore, & Bleiberg, 2001) Math, Continuous Performance Test (CPT), and Grammatical Reasoning tests (referred to collectively as the ANAM-Core), and the Psychomotor Vigilance Task (PVT). The order of tasks in the experimental protocol, starting at 1900 Friday, was always the same for all subjects; however, specific cognitive tasks, not used for ranking, were experimentally set at different practice levels prior to their fatigued measurement. These select tasks, namely SynWin, Tower of Hanoi, Manikin, and Code Substitution, had their practice histories manipulated following a simple cross-over design, in which one group's trained task set was the other group's untrained task set, and vice versa. This manipulation of training history on SynWin, Tower of Hanoi, Manikin, and Code Substitution was designed to meet an experimental objective that will not be discussed in this report.

2.3 Data Analysis

2.3.1 Main Objective

Overall (i.e., mean) sensitivity of tasks to fatigue (i.e., trial number) was assessed on best performance metrics for ANAM-core and PVT via SPSS repeated-measures MANOVAs to verify significant fatigue effects for the tasks. Subject rankings for fatigue resistance were then determined via a percent-change rule similar to that used in Chaiken, Harville, Harrison, Fischer, Fisher, and Whitmore (2008). This rule ranks subjects on percent change of cognitive performance from a baseline performance (before fatigue) to a fatigue impact measured on trials beyond the baseline (during fatigue). Because of the non-identical testing conditions between this study and the cited one (i.e., 48 hours of sleep deprivation for the cited one and 36 hours, 15 minutes for this one), adjustments to the specific ranking procedures from Chaiken et al. (2008) were made. Details on the current ranking procedure and some comparisons to the older study (both in terms of ranking methods and study results) are given in the results.

2.4 Study Events (see Appendix B. Proctor Checklists)

2.4.1 Training 1700-2030 Tuesday

Subjects received Informed Consent Documents (ICDs) prior to the Tuesday evening training. They were signed and witnessed Tuesday evening prior to the medical screening and completing the subject questionnaires. Actigraphs (i.e., wrist-worn activity monitors) were signed out with instructions. The orientation ended with instructions on dietary, medication, caffeine, exercise, and sleep/nap restrictions included in the study (see Appendix A).

Following the orientation, five replications of the ANAM-core tests and an instructional one-minute demostration of the PVT (as PVT is known to require less practice) were conducted. Additionally, integrated with the ANAM-core tasks were two replications each of trained tasks for Group 1 and 2. Group 1 was trained on SynWin and CodeSub and introduced to Tower of Hanoi and Manikin during refresher training from 1730 to 1900 the evening that sleep deprivation began (Friday). Group 2 performed these tasks in the opposite order. Each replication of a trained task was approximately three times as long as the total time for one of the three ANAM-core tests. Odd subject numbers were assigned to Group 1 and even subject numbers were assigned to Group 2.

2.4.2 Training 1700-2015 Wednesday

Subjects were first checked for compliance with the instructions on sleep, caffeine reductions, and recorded sleep log (in case the Actigraph malfunctioned). Next, four replications of ANAM-core tests were given and one complete 10 minute trial of PVT. Interleaved with the ANAM-core were three replications each of the trained tasks assigned for Group 1 and 2. Participants were then reminded of their instructions on dietary, medication, caffeine, exercise, and sleep/nap restrictions, and given instructions and equipment for their first urine sample (Friday 0700). For the initial urine sample, collection jars, pipettes, collection test tubes, and instructions (also used during the protocol) were provided. Additionally, coolers and icepacks were provided so that the initial sample could be delivered to Brooks refrigerated.

Finally, the Cattell Culture Fair Intelligence Test (Nenty & Dinero, 1981) was given at the end of training to examine issues related to the trained/untrained task, between-subjects, group manipulation. However, we predicted "no relationship" of Cattell scores to fatigue impact on the basis of observations from our earlier research (Chaiken et al., 2008) that showed fatigue resistance ranking and baseline performance (before fatigue) were unrelated. While there was no Cattell test given in the earlier study, baseline performance on the ANAM-Core tasks were unrelated to participants' fatigue classifications.

2.4.3 Friday

Participants' first urine sample was collected at 0700 and prior to having any breakfast. Participants kept a food log on Friday for what they ate for breakfast and lunch (the latter required at 1100). They were told to consume no food or beverage other than water between lunch Friday and starting the sleep deprivation later that day at 1900. Participants arrived at the testing facility at 1730, at which time they turned in their baseline urine sample, and their actigraphs and diet logs were assessed for compliance. Non-compliance meant dismissal; however, all participants were in compliance. Next, participants received 1.5 hours of refresher training and an introduction to the novel (untrained) tasks. See Appendix B, Proctor Checklists for Friday for the task-order details. The Friday pre-testing training was necessary both to refresh the tasks and introduce new tasks, and left Thursday evening free to allow the participants quality sleep before the study.

The testing/sleep-deprivation part of the protocol started at 1900 with a scheduled urine sample, dinner break, and three hours of cognitive testing before the next block started with another one-hour break. All participants took the same tests in the same order from that point on, and the four-hour block structure repeated five more times (i.e., for a total of six times) with urine sampling occurring every other break prior to any food and at the end of the protocol (1915 Saturday). Every break included a controlled meal or snack. Participants never had to eat all the food provided at a given break and could not consume any food or beverage at any other time. The cognitive test administration times were aligned to the proctor checklist schedules, with the three-hour testing ending 10 minutes before the scheduled break to provide a slack period. If a participant finished a scheduled test item early, they were given the option to play a computer game (e.g., solitare, pinball) until the next scheduled test started.

Proctors managed the administration of tests from shortcuts provided on the computer's desktop and provided subject numbers when requested by the software. In addition to the IRB required training, a minimum of one hour of training was provided to each proctor. Each proctor shift always included at least one experienced, in-house proctor.

2.5 Classification Tasks: ANAM-core and PVT Task Descriptions

ANAM-core task 1: Math. Participants responded with left or right mouse-clicks if the arithmetic expressions (e.g., 6-4-1, 6-2+3, 1+2+1) added to less than 5 (left-click) or greater than 5 (right-click). No solution was exactly 5. Expressions were in large font: the height of the font was 6% of the screen height and the length of the string was 23% of the screen width. The task was self-paced, so fast responders solved more problems. The time out was five seconds, and time outs were counted indirectly (as time wasted) against a participant's score.

ANAM-core task 2: Continuous Performance Task (CPT). This was a recognition task using single digit numbers, including zero. A stream of digits were presented (the current one overwriting the last) and participants indicated whether the current digit was the same as the digit preceding the last digit (i.e., same as the one "two back"). A very large font was used (font height 1/5 of screen height; character width about 1/11 of the screen width). If the participant did not respond, a digit would disappear after one second and would "time out" 1.5 seconds after stimulus onset. If the participant responded earlier than the timeout, a new digit would be presented one second later. Time outs were counted as errors.

ANAM-core task 3: Grammatical Reasoning. This task presented 48 symbol problems in random order each testing session. In each problem, three lines of screen text (font point-size 16) are shown. The first two lines include symbols paired with "BEFORE" or "AFTER" (e.g., * BEFORE #, & AFTER #) and the third line is a list of three different symbols (e.g., & * #), which the two preceding sentences either described correctly or incorrectly. Participants responded with a left-click, if both sentences were true or both false with respect to the third line. If one sentence was true but the other false, participants responded with a right-click. Time outs were set at 15 seconds and were counted as errors.

Psychomotor Vigilance Task (PVT). Participants took this task at the same computer station as the ANAM using the PVT-192. The PVT-192 is a portable, hand-held reaction-time device previously shown to be sensitive to sleep loss (Dinges, Pack, Williams, Gillen, Powell, Ott, Aptowicz, & Pack, 1997). The device randomly and repeatedly presented a 3-mm visual stimulus to which the participant responded by pressing a push-button with the right thumb. The inter-stimulus intervals varied from 2 to 12 seconds. The data extractor for the PVT was provided by the vendor. The data were verified by examining the summarized data. Data filtering removed very fast responses (e.g., less than 150 msec), but not very slow responses.

2.6 Research Equipment / Research Location

Test equipment was located in the Warfighter Fatigue Countermeasures Laboratory in Building 170, Brooks City-Base. This laboratory included five Dell Pentium desktops with Windows XP SP2 and 19-inch LCD monitors. These were loaded with ANAM4 (C-Shop, 2009) software and SynWin software (from the ANAM 2001 version, Reeves, Winter, Kane, Elsmore, & Bleiberg, 2001, which included a version of the SynWin task, see Elsmore, 1994). In addition, five PVT boxes and associated software were used. Urine collection containers, gloves, and related medical supplies were supplied by the Applied Biotechnology Branch (711 HPW/RHPB) at Wright Patterson AFB, OH.

3.0 RESULTS

3.1 Overview

The final recommendations for fatigue-resistance rankings (most resistant to most susceptible) for participants of the Biological Markers of Fatigue (BMOF) study are provided below. We have included a comparison of BMOF to a past fatigue-resistance ranking study, the Genetics of Sleeplessness Tolerance (GOST) Study. Next, we review the findings specific to BMOF. Finally, based on these sections, we recommend the best approaches to classifying participants with respect to fatigue resistance and provide the anonymous rankings.

3.2 Comparing Biological Markers of Fatigue to the Genetics of Sleeplessness Tolerance Study

This section describes the empirical differences and similarities between the BMOF and GOST findings in preparation for recommending BMOF fatigue-resistance rankings.

3.2.1 Attrition

Table 1 shows attrition rates from the two studies. These rates reflect only those cases that started the protocol and then dropped out for reasons of fatigue discomfort (i.e., one non-fatigue-related case of attrition in BMOF was not included in Table 1). While it has been suggested that the attrition in BMOF is proportionally higher a one-tailed test shows this is non-significant (Fisher's Exact test, $p<.14$).

Table 1. Cross-study Comparison of Subject Attrition (Failure to Complete the Protocol)

	Completed the protocol	Dropped out after starting
GOST	89	7
BMOF	23	5

3.2.2 Form of the Fatigue Function

An idealized fatigue function (i.e., as predicted from a theory of fatigue, namely, Sleep Activity Fatigue Task Effectiveness (SAFTE), described in Hursh et al. 2004) holds that performance declines steeply after midnight until mid-morning where it levels off to a long plateau of stable performance until the next decline after midnight. While GOST subjects conformed to this pattern, BMOF subjects showed the expected decline after midnight, but had unanticipated improvements toward the end of the protocol Saturday at 1915. This is often attributed to a going-home effect; however, it is an effect in BMOF that persisted a significant percentage of the last part of the protocol (i.e., 3 hours). It is also possible to interpret the effect as an increased circadian-nadir effect in the early morning.

To show a GOST / BMOF comparison in the fatigue functions, the score of each subject's performance trials on ANAM core tasks (Math, Grammatical Reasoning, and Continuous Performance Task) was converted to intrasubject *z* units. This allowed us to average participants' ANAM scores together and compare an aggregate (z) ANAM function across studies. An intrasubject z-transformation is not a standard z transformation across all subjects, but a z-transformation done completely **within** a subject for each task (see Chaiken, et al., 2008, for details). Figure 1 plots the fatigue impacts of GOST and BMOF over a comparable number of trials and range of sleep deprivation (i.e., the first 12 testing trials of GOST and all 12 testing trials of BMOF). Each mean point is bracketed by a 4 standard error (s.e.) of the mean. For ANAM tasks, positive *z* scores reflect better performance than negative *z* scores. A MANOVA on the trial data displayed in the figure shows a significant interaction of Study x Time ($F(11,1199)=11.21$, $p<.0001$). Of course, there are other differences between the studies beside the length of sleep deprivation. For instance, the BMOF function is an hour later relative to the GOST function (i.e., BMOF started the first testing trial at 2000 rather than 1900). GOST also had tasks not used for classification that BMOF did not have and vice versa. However, the most plausible explanation for the differences in the functions is related to the fact that the BMOF subjects knew the end was near at trial 11, whereas at trial 11 the GOST subjects knew that 14 more hours were remaining.

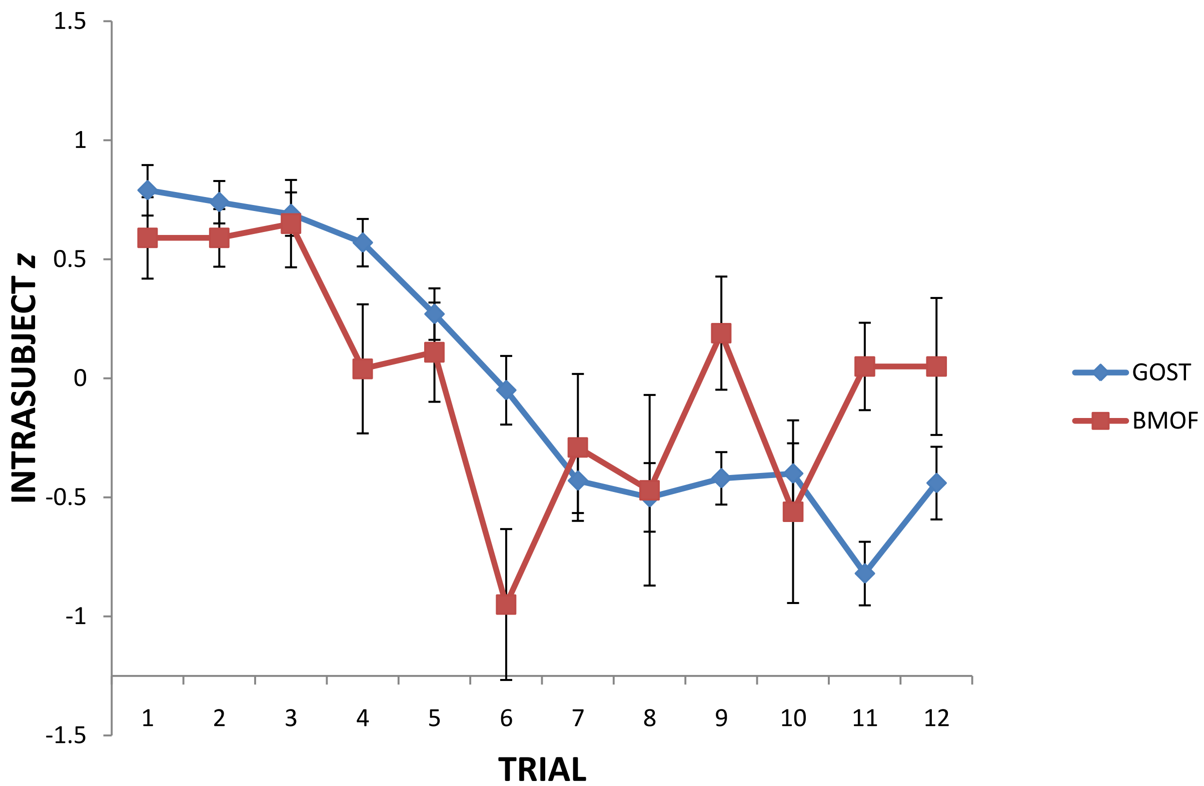

Figure 1. Fatigue functions for composite (z) ANAM tasks compared across two studies

In BMOF, the "going-home effect" was less evident with the Psychomotor Vigilance Task (PVT), which was always administered after every ANAM core task set. PVT data consisted of false starts, reaction times to stimulus onset, and the number of lapses (or failures to respond). The scoring metric for PVT was lapses + false starts. Figure 2 compares GOST and BMOF PVT performance. Positive *z* scores reflect poorer performance than negative *z* scores, and error bars are again 4 s.e. brackets.

A MANOVA analysis showed the Study x Time interaction was significant ($F(5,550)=3.18$, $p<.008$), although its interpretation is less clear. While the functions between studies are similar, the PVT was administered six times in the first 24 hours of sleep deprivation in GOST, it was administered 12 times during the same period in BMOF. Therefore, while the plot reflects PVT trials at similar administration times, the PVT was conducted twice as frequently in BMOF.

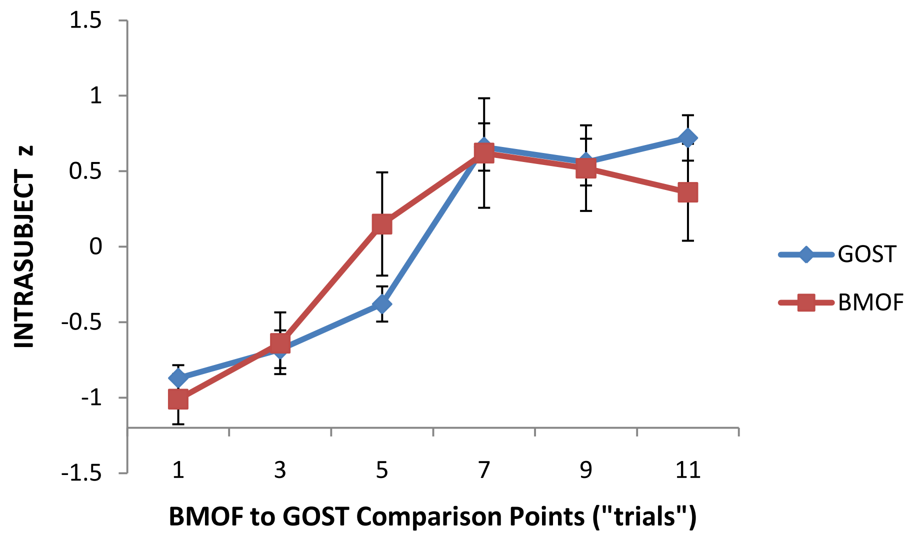

Figure 2. Fatigue functions for the PVT task compared across two studies

3.2.3 Comparison of Initial (Rested) Ability across Studies

The initial three testing trials after training for each ANAM task provided estimates of the subjects' practiced aptitude for the tasks prior to sleep deprivation. Comparing these across studies checks for population differences between BMOF and GOST. Table 2 compares the earliest PVT testing trial from each study. There was no systematic or significant difference for task aptitude between studies.

Table 2. Cross-study Comparison of Initial Testing-Trial Ability for Fatigue Classification Tasks

	Math	Grammatical Reasoning	CPT	PVT
GOST	56	87.4	86.0	4.32
BMOF	51	90.2	91.7	3.09
***t*(110); 2-tailed significance**	1.83 p<.07	-1.21 p<.23	-1.87 p<.06	.93 p<.355

Note. For Math the metric used was (right – wrong). The metric used for both Grammatical Reasoning and CPT was percent correct. The PVT metric was lapses + false starts.

3.2.4 Relation of Initial (Rested) Task Ability to Fatigue Classification

When we consider the relationship between a subject's ability *for a task* and their relative fatigue resistance ranking *with respect to that task*, we are considering hypothetical desiderata of a fatigue resistance measurement, namely that fatigue resistance should be measured in such a way that it is not task dependent. In GOST, the subject rested ability to perform tasks was fairly independent of their fatigue-resistance ranking (see Chaiken, et al., 2008, Figures 3 and 4 and the beginning of the discussion).

In BMOF, each subject was given a fatigue-resistance ranking for each task. Specifically, for ANAM tasks, the average of a subject's first three testing trials was their baseline or non-fatigued performance for that task. The average of their next nine testing trials was used to determine the fatigue impact for that task. The difference was the percent change using the following formula : ((Fatigue Impact)-Baseline)/Baseline. For PVT, the first two complete (i.e., 10 minute long) *training* trials Wednesday and Friday had better performance on average than any subsequent testing trial, so baseline PVT was the average of the two complete PVT training trials. Since PVT could have baseline scores of 0.0, we used simple-change as the fatigue impact metric for that task (as in GOST). A participant's fatigue ranking on PVT was defined as: (PVT Fatigue Impact) – (PVT Baseline).

Table 3 shows the result of evaluating fatigue-resistance / rested-task-ability relationships in BMOF, where rested-task ability is defined as the performance baseline described above. Fatigue resistance classification within a task was accomplished by ordering subjects on either their percent-change or simple-change fatigue impact (ANAM and PVT, respectively) for a task, and dividing the ordered sort roughly in half (i.e., n=23). In all cases where "median-split" is referenced for subject rankings in this report, the split was accomplished by calling the best 11 (i.e., on the "good" side of the median) as resistant and the worst 12 (i.e., on the "bad" side) as susceptible. Table 3 shows that for BMOF, a lower rested ability for a task generally goes with greater fatigue susceptibility on that task (with the exception of Math).

While the tests assessed are identical to GOST, the method of fatigue-resistance ranking for GOST and for BMOF are necessarily different, owing to the different amounts of sleep deprivation given in the two studies (i.e., GOST: 48 hours; BMOF: 36-hours, 15 minutes). For GOST, the fatigue-resistance ranking was percent-change from the baseline of the first four trials to a fatigue endpoint of the last four trials. The GOST method disregarded 10 intervening testing trials between the baseline and fatigue endpoint. GOST also created a fatigue-resistant classification on the basis of all three ANAM tasks by averaging the percent-change ranks for each of them to form an aggregate percent change rank for a subject. This aggregation of ANAM tasks (to form one classification from all of the ANAM) *was not pursued* in BMOF for the reasons explained in the final recommendation section.

Table 3. Rested-Task Ability in BMOF as it Relates to Susceptible/Resistant Classification

	Math	Grammatical Reasoning	Continuous Performance	Psychomotor Vigilance Task
Classified susceptible (n=12) on task by median-split	51.2	86.7	88.9	3.25
Classified resistant (n=11) on task by median-split	51.6	94.1	94.7	1.68
t(21) ; 2-tailed significance	0.1 ; ns	-2.55 ; p<.02	-2.39 ; p<.03	2.2 ; p<.04

Note. For Math the metric used was (right – wrong). The metric used for both Grammatical Reasoning and for CPT was percent correct. The PVT metric was lapses + false starts.

3.3 Results Unique to the BMOF Study

3.3.1 Participant Characteristics

Demographic information was collected using self-report forms on Tuesday. Nineteen males and four females completed the BMOF protocol. Seventeen were current or past military members, and six were civilian. Mean age was 27.0 years with a standard deviation (SD) of 4.4 years. Mean weight was 168.5 lbs. (SD 28.9), mean height was 69.7 inches (SD 3.2), mean habitual weekday sleep was 7.4 hrs. (SD .70 hrs), mean habitual weekday bed time was 2130 (SD 45 min), mean habitual weekday wake-up time was 0624 (SD 1 hr 27 mins), mean habitual weekend sleep was 8.2 hrs (SD 1.0 hr), mean habitual weekend bed time 2327 (SD 1.0 hr), and mean habitual weekend wake-up time was 0809 (SD 1 hr 36 mins). We revisit the sleep characteristics after we recommend fatigue resistance rankings. At that point, we will assess whether recommended fatigue-resistance rankings related to any of these self-reported sleep characteristics.

Participants also varied with respect to a between-subjects factor unrelated to our classification tests. Specifically one group of twelve differed from the other group of eleven with respect to which *non-classification* tests they had received practice on before sleep deprivation, and which other non-classification tests they were introduced to just prior to sleep deprivation. This factor was assessed for possible effects on the fatigue impact for each task used to classify participants and the Cattell test. As expected, no effect was found.

3.3.2 Performance Decline with Trial Number on Candidate Fatigue-Resistance Classification Tasks

Table 4 shows the basic fatigue results with respect to four candidate tasks that could be used to classify BMOF participants. That is, these tasks were "pre-practiced" to the same extent by every participant prior to the testing trials under sleep deprivation. As previous studies have shown, the effect of trial hours into the protocol on performance is significant, but the maximum fatigue impact was not at the end of the protocol, but more towards the middle. Table 4 also shows that *enough* practice was provided for each task so that the performance baselines reflect both maximum and stable performance.

Table 4. Basic BMOF Results for Candidate Fatigue-Resistance Classification Tasks

	Math		Grammatical Reasoning		Continuous Performance		Psychomotor Vigilance	
	mean	sd	mean	sd	mean	sd	mean	sd
Training	50.3	6.9	89.2	8.4	88.8	10.0	2.5	1.9
Trial 1 (2015)	51.4	9.3	89.8	9.5	92.7	5.3	3.1	4.9
Trial 2 (2230)	51.6	10.7	89.7	9.1	90.0	9.8	6.5	7.6
Trial 3 (0015)	51.3	10.0	91.2	7.2	92.4	6.6	6.3	8.0
Trial 4 (0230)	47.1	10.3	83.5	17.8	86.0	12.8	10.7	13.7
Trial 5 (0415)	46.9	9.4	84.1	15.9	87.0	13.9	13.6	10.2
Trial 6 (0630)	39.9	13.8	76.1	14.4	73.3	20.2	20.1	14.1
Trial 7 (0815)	47.0	9.0	78.6	16.6	84.0	11.9	15.6	11.4
Trial 8 (1030)	43.2	15.5	77.2	19.2	76.8	20.7	14.9	9.5
Trial 9 (1215)	48.9	8.8	86.7	11.3	88.2	9.2	15.3	8.5
Trial 10 (1430)	44.3	14.7	76.7	18.4	77.5	18.2	13.1	9.5
Trial 11(1615)	46.4	12.2	83.5	15.4	86.0	12.3	15.4	13.1
Trial 12(1830)	46.9	13.1	84.2	18.6	84.8	12.9	8.7	6.6
Trial Effect: *F*(11,242); *p*-value	5.37; .0001		7.25; .0001		9.51; .0001		7.66; .0001	

Note. A trial's administration time (computed as the midpoint time between the start of ANAM and finishing the PVT) is given in parentheses after trial number. Trials averaged to obtain a baseline performance on a task are highlighted in green. Trials highlighted in yellow reflect the three worst scores (on average) for a task. For Math the metric used was (right – wrong). The metric used for both Grammatical Reasoning and for CPT was percent correct. The PVT metric was lapses + false starts. The mean of 2.5 for PVT during training is for Wednesday at 1900 and during Friday's refresher training at 1745.

3.3.3 Fatigue Impacts: Correlated Across Tasks and to the Cattell Culture Fair Test of Intelligence

Percent-change from fatigue for the three ANAM tasks and simple change from fatigue for the PVT were correlated to each other and to performance on the Cattell Test. The results are shown in Table 5.

Table 5. Intercorrelations of Task Fatigue Impact and the Cattell Test

	I.	II.	III.	IV.
I. Math Processing				
II. Continuous Performance	.55 (.007)			
III. Grammatical Reasoning	.48 (.022)	.77 (.0001)		
IV. Psychomotor Vigilance	.33 (.127)	.38 (.076)	.30 (.162)	
V. Cattell Culture Fair	-.27 (.212)	-.51 (.014)	-.82 (.0001)	-.23 (.287)

Note. N = 23 for correlations. A two-tailed significance level is in parentheses. Variable V. is the total correct from Scales 2 and 3 of the Cattell Culture Fair Test (Form A).

Table 5 shows the Cattell score as being substantially correlated with fatigue impact for the Grammatical Reasoning Test and moderately with the Continuous Performance Task. Table 5 also indicates that while Math and PVT fatigue impacts are not significantly related to Cattell, they are also not significantly related to each other. Table 6 better shows this last point with the breakdown of subjects classified as resistant/susceptible on the Math task crossed by those classified as resistant/susceptible on the PVT task (via median splits on their respective fatigue impact measures). While the correct diagonal has the most observations, there is also a fair amount of disagreement evident (as expected with a non-significant correlation).

Table 6. Fatigue Resistance Classification Agreement between ANAM Math and PVT

	Susceptible: PVT	Resistant: PVT
Susceptible: Math	7	5
Resistant: Math	5	6

3.4 Discussion

Although not shown in a table, both baseline and non-baseline cognitive performance scores correlate to the Cattell scores. This is expected given all the cognitive tasks are inter-related to some degree. However, the expectation for a relationship between a person's fatigue impact and their general intelligence is more of an open issue. In the particular case of BMOF, a very strong relation between Cattell and fatigue impacts on Grammatical Reasoning, but not on Math is a complication. This finding suggests there are multiple determinants of fatigue impact, which would make the search for predictive or correlative biomarkers of fatigue more complicated. We make an assumption that it is the fatigue impact that is not related to general (rested) intelligence that is the desired fatigue impact to assess further.

Although Cattell was not a part of GOST, the fatigue impact scores for that study could be expected to correlate less with Cattell. This is plausible given fatigue impact scores in GOST were relatively far more removed from the baseline scores (i.e., 10 intervening testing trials). This might suggest that tactics more similar to GOST could be adopted to define fatigue impact in BMOF, for example, by looking at the last trials for fatigue impact. However, the sleep deprivation in GOST was greater and the "going-home" effect in BMOF would make this tactic misleading. One could also propose that some function of the worst performance(s) after baseline as a fatigue endpoint by which percent-change (or simple change for the PVT) could be computed. However, this would discount other fatigued performance as unimportant and would weigh potentially transient lapses in motivation as being more important to classification. We favor defining fatigue impact as the average of all non-baseline scores, precisely because that method weighs all characterizations of fatigue impact impartially.

Although the ANAM Continuous Performance Task has a fatigue impact moderately correlated with Cattell, the connection is really much weaker than the similar one found for Grammatical Reasoning. T-tests comparing above and below-median Cattell scorers on their Continuous Performance fatigue impact were not significant. The ANAM Math and ANAM CPT could be combined into a single fatigue impact ranking by averaging their separate rankings together. We are ambivalent on this suggestion as CPT fatigue impact scores correlate lower to Math's fatigue impact scores than they do to Grammatical Reasoning's fatigue impact scores (and we know the latter's fatigue impact scores are strongly related to Cattell). We also found baseline performance on Continuous Performance was more significantly related to a participant's fatigue-resistance classification on that task; whereas, Math's baseline performance was not related to its fatigue-resistance classification.

3.5 Ancillary Data

Table 7 provides rankings on Grammatical Reasoning and Continuous Performance. Table 8 provides Cattell Scores to support using the other two tasks for fatigue classification and correcting for Cattell (although this is not necessarily recommended). One could also do an analysis of Cattell alone as a non-fatigue-related participant characteristic that could be related to biomarkers.

Figures 3 and 4 plot the fatigue functions for resistant and susceptible participants using Grammatical Reasoning and Continuous Performance. Some trends in these ANAM fatigue functions suggest that fatigue-susceptible performance greatly improved after meals (i.e., Trials 7 and 9 occur after a breakfast and lunch, respectively). This trend also occurs for Grammatical Reasoning, but only for Trial 9, and possibly for PVT, but only for Trial 7. Meals occurred during the daylight portion of the protocol with greater frequency than the night time portion; however, participants were allowed standardized, limited snacks during non-meal breaks.

Table 7. ANAM Grammatical Reasoning and Continuous Performance Fatigue Rankings

ID	Grammatical Reasoning Percent-Change Fatigue Impact Score	Median-Split Classification
K	-4.01	1
A	0.24	1
W	1.79	1
P	2.93	1
E	3.65	1
M	3.7	1
D	3.85	1
J	4.49	1
C	5.36	1
F	7.33	1
N	7.35	1
R	7.98	0
B	9.07	0
I	9.18	0
V	11.2	0
H	14.95	0
Q	15.06	0
G	16.14	0
O	17.87	0
L	21.24	0
S	24.35	0
U	27.84	0
T	31.82	0

ID	Continuous Performance Percent-Change Fatigue Impact Score	Median-Split Classification
F	0	1
A	0.25	1
K	2.62	1
W	3.91	1
D	4.24	1
J	4.89	1
O	4.94	1
C	5.44	1
R	5.76	1
I	6.52	1
Q	7.13	1
B	7.93	0
E	8.16	0
N	9.19	0
P	9.58	0
H	9.78	0
G	9.85	0
L	10.97	0
V	11.1	0
M	16.65	0
S	18.48	0
T	37.16	0
U	37.83	0

*Note. M*edian-split: 1=resistant; 0=susceptible.

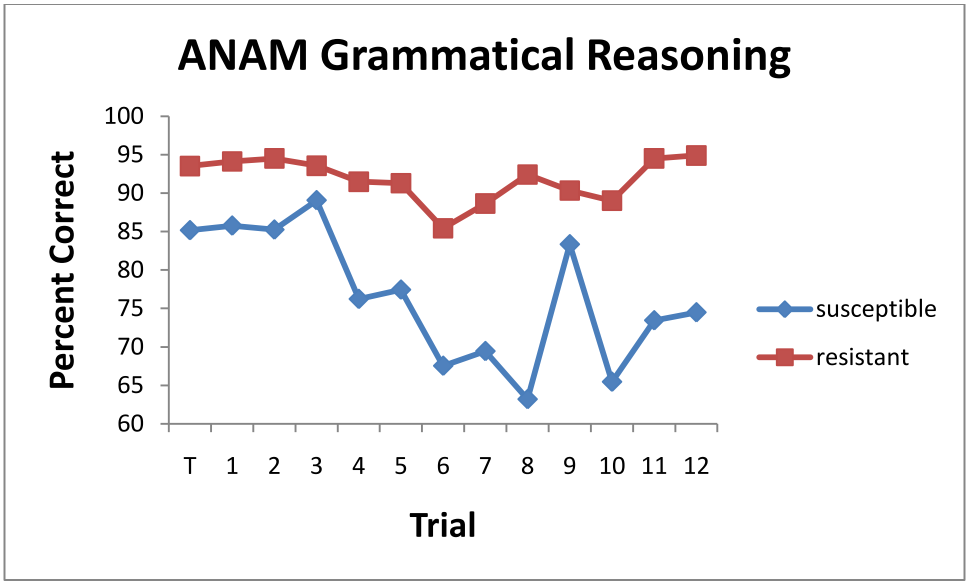

Figure 3. Plot of median-split groups from Table 7 (Grammatical Reasoning) for average training performance (T) and testing trials (1-12)

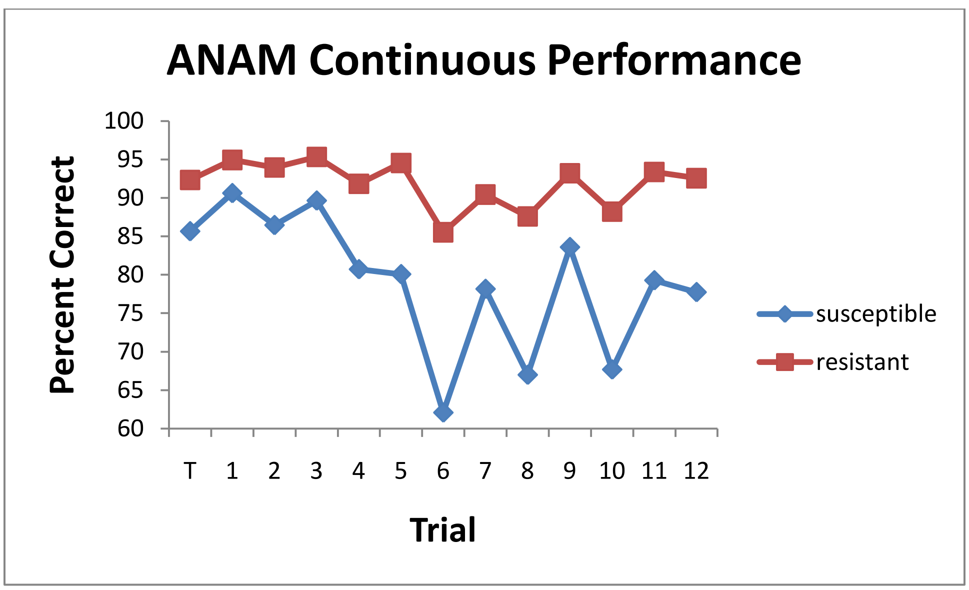

Figure 4. Plot of median-split groups from Table 7 (Continuous Performance) for average training performance (T) and testing trials (1-12)

Table 8. Subject Rankings on Cattell

ID	Cattell Total Score	Cattell Median Split
S	54	0
T	59	0
Q	59	0
O	59	0
U	59	0
I	65	0
L	66	0
D	66	0
G	68	0
B	68	0
F	71	0
H	71	0
W	73	1
R	73	1
V	73	1
A	75	1
M	75	1
E	76	1
J	76	1
N	79	1
C	79	1
P	80	1
K	81	1

Note. Total score is (Scale 2 + Scale 3). Median-split: 1=resistant; 0=susceptible.

3.6 Recommendations and Rankings

Given the findings above, we **strongly recommend** PVT be used as the primary classifier owing to its prominence and accuracy in the fatigue literature (see Lim and Dinges, 2008). The only negative with PVT with regards to classification is that its baseline performance levels appeared to not relate to fatigue classification in this study as much as some of the ANAM-core tasks (see Table 3). As fatigue impact on the PVT was unrelated to performance on the Cattell test, this negative aspect for PVT is weakened even further. When a secondary classifier is needed, we recommend Math (which does not have such prominence in the fatigue literature) as the secondary classifier of fatigue resistance. Math shows clear advantages over Grammatical Reasoning and Continuous Performance in terms of isolating an independent trait with respect to fatigue-resistance (i.e., not determined by a person's fatigue-irrelevant characteristics, such as intelligence). However, we cannot recommend aggregating PVT and Math together as the basis of a single classification. The two methods of classification do not correlate very highly (see Table 6). We made a similar observation and recommendation in GOST and there is literature suggesting PVT fatigue impact may be different from the fatigue impact observed on cognitive tasks (see the factor analysis in Van Dongen et al., 2004).

3.6.1 Final Assessment: Fatigue Classification Related to Reported Sleep Behaviors

The results for the PVT and for Math tests can be analyzed for correlations to the participants' reported sleep behaviors. Table 9 provides the data for both sets of rankings using questionnaire data provided Tuesday evening during training. In general, reported sleep behavior does not relate to fatigue resistance rankings for these two tasks. The one near miss in the table, that is, $t(21)=2.06$ ($p<.052$; two-tailed) is in the wrong direction with respect to explaining fatigue-resistance as a side effect of better sleep hygiene. That is, it is the resistant group that reports waking up earlier, which should put them at a disadvantage relative to the susceptible group. One possible outlier is participant "H" who reported habitual wake up times on weekdays at 1130 (despite habitual bed times as 2330 and habitual sleep amounts as 9 hours). If the wake-up times are not errors in filling out the form, then this participant may be excluded from further analyses (on the basis of having anomalous sleep patterns); however, exclusion of this participant will not make any of the results shown in Table 9 significant. Note that the actual subject IDs used to collect all data were numbers. To further protect subject anonymity after all data were collected and analyzed, starting in Table 10 subject IDs were assigned "A" (most fatigue resistant subject on PVT) to "W" (the least fatigue resistant subject on PVT). None of the actual subject number IDs used for data collection can be matched to the corresponding "A" to "W" subject IDs by reading the current report. Figure 5 plots the median split on PVT, and Table 11 and Figure 6 are the Math median split.

Table 9. Historical Sleep Behaviors Self-reported During Training Compared Across Post-hoc Susceptible/Resistant Classifications

	Weekday Sleep per night (hours)	Weekday Bedtime	Weekday Waketime	Weekend Sleep per night (hours)	Weekend Bedtime	Weekend Waketime
Susceptible PVT	7.33	2230	0600	8.0	2330	0752
Resistant PVT	7.50	2236	0648	8.5	2324	0827
t(21)	-.56	-.42	-1.33	-1.16	.25	-.87
Susceptible Math	7.50	2238	0658	8.17	2328	0808
Resistant Math	7.32	2227	0546	8.32	2326	0811
t(21)	.61	.54	2.06	-.34	.06	-.08
2-tailed significance	p<.55	p<.60	p<.052	p<.74	p<.95	p<.93

Note. Susceptible vs. Resistant is determined by median split on simple-change and percent-change rankings for PVT and Math, respectively.

Table 10. PVT Fatigue Rankings

ID	PVT Simple Change Fatigue Impact Score	Median Split Group Classification
A	2.33	1
B	2.42	1
C	4.42	1
D	5.08	1
E	5.5	1
F	5.92	1
G	6.25	1
H	6.33	1
I	6.5	1
J	6.75	1
K	6.92	1
L	8.83	0
M	8.92	0
N	9.33	0
O	10.42	0
P	11.42	0
Q	12.17	0
R	12.58	0
S	12.75	0
T	14.17	0
U	15	0
V	19.33	0
W	23.75	0

*Note. D*ata collection IDs were numbers, not letters. Median-split: 1=resistant; 0=susceptible.

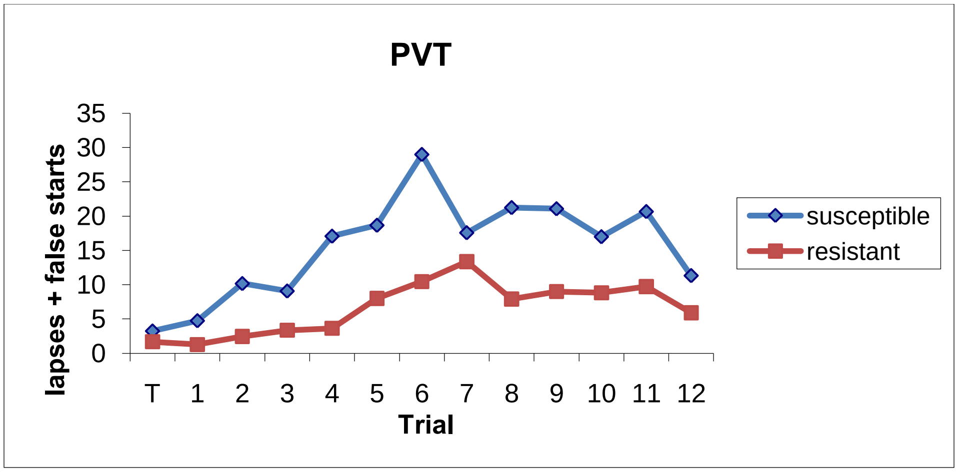

Figure 5. Plot of median-split groups from Table 10 (PVT) for average training performance (T) and testing trials (1-12)

Table 11. ANAM Math Fatigue Rankings

ID	Math Percent Change Fatigue Impact Score	Median Split Group Classification
D	-9.91	1
F	-7.67	1
M	1.19	1
C	2.52	1
Q	4.65	1
A	5.31	1
E	5.38	1
L	6.93	1
T	8.09	1
J	8.66	1
P	9.43	1
O	9.52	0
K	11.68	0
W	11.74	0
I	12.53	0
V	14.79	0
N	15.02	0
B	17.76	0
R	19.73	0
H	20.62	0
S	21.12	0
G	22.01	0
U	48.06	0

*Note. M*edian-split: 1=resistant; 0=susceptible.

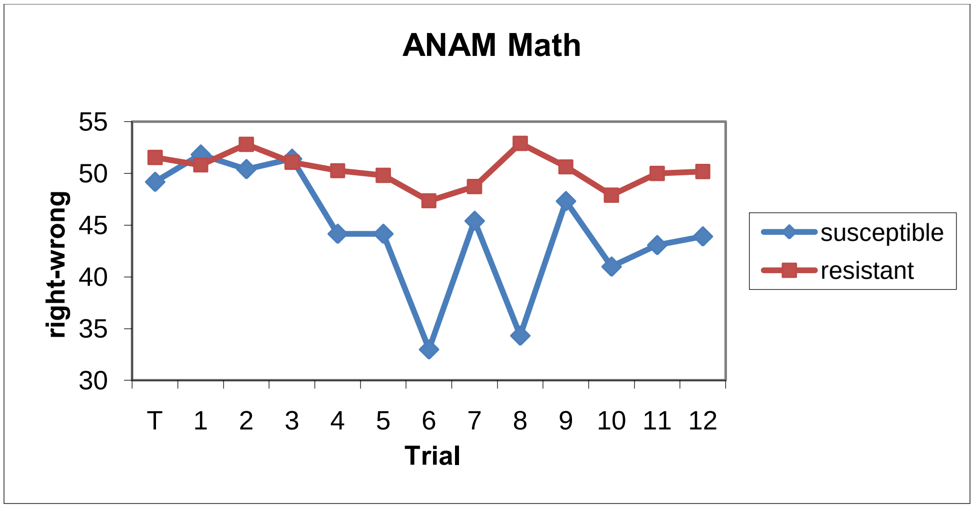

Figure 6. Plot of median-split groups from Table 11 (Math) for average training performance (T) and testing trials (1-12)

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LIST OF ACRONYMS AND ABBREVIATIONS

ANAM (Automated Neuropsychological Assessment Metrics)

BMOF (Biological Markers of Fatigue)

CPT (Continuous Performance Test)

GOST (Genetics of Sleeplessness Tolerance)

ICC (intraclass correlation coefficient)

ICD (informed consent document)

IRB (Institutional Review Board)

LC (liquid chromatography)

LDA (linear discriminant analysis)

MS (mass spectrometry)

NMR (nuclear magnetic resonance)

PCA (principal component analysis)

PVT (Psychomotor Vigilance Task)

SAFTE (Sleep Activity Fatigue Task Effectiveness)

SD (standard deviation)

T (training)

APPENDIX A. STUDY RESTRICTIONS

SLEEP AND SCHEDULE	Prior to the week of the study
Sleep Habits	For at least 30 days prior to the study, you should be sleeping between the hours of 11pm and 7am +/- 1 hour (i.e., earliest times sleep/wake: 10pm to 6am, latest times sleep/wake: midnight to 8am.) For example, if you usually sleep from 1am to 9am, you cannot participate in this study.
Work Schedule	Up to 30 days prior to the study, maintain work hours within the 7am to 6pm time period.

	Week of the study (release is 7:15PM Sat)		
DIETARY RESTRICTIONS	**Wed**	**Thurs**	**Fri & Sat**
Caffeinated Food/Drink (e.g. coffee, cola, chocolate)	**reduce by 1/3**	**reduce by 1/3**	**NONE** after Thurs midnight until release
Nicotine (e.g. tobacco products)	**NO**	**NO**	**NO**
Medication (over the counter or prescription)	**OK**	**NONE** after 7:00 AM	**NONE** until release
Alcoholic beverages, fish, cheese, garlic, onion, beetroot, asparagus, cherries, grapefruit, or liquorice	**OK**	**NONE** after 7:00 AM	**NONE** until release
Food or beverage NOT provided by us	**OK** (we provide NO food)	**OK** (we provide NO food)	**NONE** after 11:00AM until release (we provide food during your time at Brooks)
Chewing gum	**OK**	**OK**	**NONE** after 11:00AM until release

SLEEP/NAP REQUIREMENTS	Tues Night / Weds Morn	Wed Night / Thur Morn	Thurs Night / Fri Morn
Amount of sleep	**at least 7 hours**	**at least 7 hours**	**at least 7 hours**
Wake time	**by 8:00 AM**	**by 8:00 AM**	**MUST WAKE** AT **7:00 AM**
Naps	**OK** (Wednesday)	**OK** (Thursday)	**NONE** after waking at 7:00AM Fri

OTHER ACTIONS	Tues Night and Wed	Wed Night and Thurs	Thurs Night and Fri
Exercise	**OK**	**OK**	No strenuous exercise after 7PM THURS NIGHT and throughout the study period.
Actigraph (must be worn except while showering or swimming)	All Night and Day	All Night and Day	All Night and Day (MUST be turned in 5:30PM Friday)
Short Sleep Log	Answer questions	Answer questions	Answer questions and bring to Brooks
Urine Sampling	NONE	NONE	7:00AM FRIDAY MORN **BEFORE EATING**; Keep in provided cooler and bring to Brooks
Periods in which YOU CANNOT EAT (but can drink water or 0 calorie beverage)	NONE	NONE	**Friday after 11:00AM (lunch) until 7:00PM**
Keeping a FOOD LOG (notepad provided to you Weds Night)	NONE	NONE	From 7:00AM Friday until release

After being released, do NOT drive or operate machinery before having adequate sleep.

APPENDIX B. PROCTOR CHECKLISTS

Tuesday Evening Training ODD # Subjects		
√	Start Time	Task
	1700	Intro/ICD/ID Check/Med Screening
	1720	Actiwatch Instructions
	1740	Sleep History & Demography Forms
	1800	ANAM-Core INSTRUCT
	1825	SynWin INSTRUCT
	1840	ANAM-core
	1855	Code Substitution INSTRUCT
	1910	ANAM-core
	1925	SynWin
	1940	ANAM-core
	1955	Code Substitution
	2010	ANAM-core
	2025	PVT DEMO
	2030	Finish

Tuesday Evening Training EVEN # Subjects		
√	Start Time	Task
	1700	Intro/ICD/ID Check/Med Screening
	1720	Actiwatch Instructions
	1740	Sleep History & Demography Forms
	1800	ANAM-Core INSTRUCT
	1825	Tower of Hanoi INSTRUCT
	1840	ANAM-core
	1855	Manikin INSTRUCT
	1910	ANAM-core
	1925	Tower of Hanoi
	1940	ANAM-core
	1955	Manikin
	2010	ANAM-core
	2025	PVT DEMO
	2030	Finish

Wednesday Evening Training ODD # Subjects		
√	Start Time	Task
	1700	Compliance Check
	1705	ANAM-core
	1715	SynWin
	1730	ANAM-core
	1745	Code Substitution
	1800	ANAM-core
	1815	SynWin
	1830	ANAM-core
	1845	Code Substitution
	1900	PVT
	1910	SynWin
	1925	Code Substitution
	1940	Cattell Culture Fair Test
	2010	Urine Sample Instructions
	2015	Finish

Wednesday Evening Training EVEN # Subjects		
√	Start Time	Task
	1700	Compliance Check
	1705	ANAM-core
	1715	Tower of Hanoi
	1730	ANAM-core
	1745	Manikin
	1800	ANAM-core
	1815	Tower of Hanoi
	1830	ANAM-core
	1845	Manikin
	1900	PVT
	1910	Tower of Hanoi
	1925	Manikin
	1940	Cattell Culture Fair Test
	2010	Urine Sample Instructions
	2015	Finish

Friday Training ODD # Subjects		
√	Start Time	Task
	1730	Compliance Check/ANAM-core
	1745	PVT
	1800	SynWin
	1815	Tower of Hanoi INSTRUCT
	1830	Code Substitution
	1845	Manikin INSTRUCT
	1900	Urine/Dinner/Break

Friday Training EVEN # Subjects		
√	Start Time	Task
	1730	Compliance Check/ANAM-core
	1745	PVT
	1800	SynWin INSTRUCT
	1815	Tower of Hanoi
	1830	Code Substitution INSTRUCT
	1845	Manikin
	1900	Urine/Dinner/Break

Biomarkers Testing Schedule (Friday and Saturday)

Block	√	Start Time	Task
1		1900	Urine/Dinner/Break
		2000	ANAM-core
		2020	PVT
		2030	SynWin
		2040	Tower of Hanoi
		2055	Code Substitution
		2110	Manikin
		2125	SynWin
		2135	Tower of Hanoi
		2150	Code Substitution
		2205	Manikin
		2220	ANAM-core
		2240	PVT
2		2300	Break
		2400	ANAM-core
		0020	PVT
		0030	SynWin
		0040	Tower of Hanoi
		0055	Code Substitution
		0110	Manikin
		0125	SynWin
		0135	Tower of Hanoi
		0150	Code Substitution
		0205	Manikin
		0220	ANAM-core
		0240	PVT
3		0300	Urine/Break
		0400	ANAM-core
		0420	PVT
		0430	SynWin
		0440	Tower of Hanoi
		0445	Code Substitution
		0510	Manikin
		0525	SynWin
		0535	Tower of Hanoi
		0550	Code Substitution
		0605	Manikin
		0620	ANAM-core
		0640	PVT

Block	√	Start Time	Task
4		0700	Break/Breakfast
		0800	ANAM-core
		0820	PVT
		0830	SynWin
		0840	Tower of Hanoi
		0855	Code Substitution
		0910	Manikin
		0925	SynWin
		0935	Tower of Hanoi
		0950	Code Substitution
		1005	Manikin
		1020	ANAM-core
		1040	PVT
5		1100	Urine/Lunch/Break
		1200	ANAM-core
		1220	PVT
		1230	SynWin
		1240	Tower of Hanoi
		1255	Code Substitution
		1310	Manikin
		1325	SynWin
		1335	Tower of Hanoi
		1350	Code Substitution
		1405	Manikin
		1420	ANAM-core
		1440	PVT
6		1500	Break
		1600	ANAM-core
		1620	PVT
		1630	SynWin
		1640	Tower of Hanoi
		1655	Code Substitution
		1710	Manikin
		1725	SynWin
		1735	Tower of Hanoi
		1750	Code Substitution
		1805	Manikin
		1820	ANAM-core
		1840	PVT
		1900	Urine and Debrief
End		1915	Release Subjects